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(54) NUCLEAR FACTOR KAPPA B ACTIVATION INHIBITOR TARGETING TAK1, AND IDENTIFICATION THEREOF

(57)Abstract:
PROBLEM TO BE SOLVED: To identify or screening NF-κB activation inhibitor usable for treatment or the like of autoimmune disease by examining the modulating activities of a specimen to the function of TGF-β activated kinase 1(TAK1).
SOLUTION: NF-κB(Nuclear Factor kappa B) activation inhibitor useful as a therapeutic agent, prophylactic or the like of autoimmune disease or intractable disease providing inflammatory symptom is identified or screened by examining the modulating activities of a specimen to the function of the TAK1. Preferably, the function of the TAK1 is the one selected from the interaction of the TAK1 and a TAK1-binding protein 1, activation of IKK complex by the TAK1 in a cell, the NF-κB activation induced by the TAK1, and the like.

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CLAIMS

[Claim(s)]
[Claim 1] The identification approach or the screening approach including the process which authorizes a modulation operation of the examined substance to the function of TAK1 (TGF-beta AKUCHIBETEDDO kinase 1) of NF-kappa B activation depressant.
[Claim 2] The approach according to claim 1 a modulation operation of an examined substance is an operation which checks or controls the function of TAK1.
[Claim 3] The approach according to claim 2 the functions of TAK1 are an interaction with (1) TAK1 and TAK1 binding protein 1, the protein kinase activity of (2) TAK1, and the thing chosen from activation of the IKK complex by intracellular TAK1, and the NF-kappa B activation guided by (3) (4) intracellular TAK1.
[Claim 4] The approach according to claim 2 the function of TAK1 is the protein kinase activity of TAK1.
[Claim 5] The approach according to claim 2 the function of TAK1 is activation of the IKK complex by intracellular TAK1.
[Claim 6] The approach according to claim 1 of including the process which makes the cell for a trial coexist with an examined substance using the cell for a trial which carried out manifestation enhancement of the TAK1 and TAK1 binding protein 1.
[Claim 7] The approach of claim 1-6 given in any 1 term which is the remedy and/or prophylactic of an intractable disease to which NF-kappa B activation depressant presents an autoimmune disease or an inflammation symptom to coincidence.
[Claim 8] NF-kappa B activation depressant chosen or identified by the approach of claim 1-6 given in any 1 term.
[Claim 9] NF-kappa B activation depressant which uses as a principal component the drug which modulates the function of TAK1.
[Claim 10] The identification approach or the screening approach of the remedy of the intractable disease which presents an autoimmune disease or inflammation symptom including the process which authorizes a modulation operation of the examined substance to the function of TAK1 in NF-kappa B activating pathway, and/or a prophylactic.
[Claim 11] The remedy and/or prophylactic of an intractable disease which present the autoimmune disease or inflammation symptom chosen or identified by the approach of claim 10.
[Claim 12] The remedy and/or prophylactic of an intractable disease which present the autoimmune disease or inflammation symptom which uses as a principal component the drug which has the operation which checks or controls the function of TAK1 in NF-kappa B activating pathway.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the remedy and prophylactic of NF-kappa B activation depressant and an autoimmune disease, and the intractable disease that presents an inflammation symptom. Moreover, it is related with those new screening approaches and the identification approach.
[0002]
[0003]
[Description of the Prior Art] NF-kappa B (Nuclear Factor kappa B) known as one of the transcription factors has played the important role in the transcriptional control of the various genes which participate in inflammation or an immune response. Usually, although NF-kappa B exists in intracytoplasmic as inactive complex combined with IkappaB-which is control protein, if a fixed stimulus is given to a cell, IkappaB will receive qualification and decomposition and it will be activated by shifting from complex. Thus, activated NF-kappa B shifts into a nucleus, combines with the unique base sequence (NF-kappa B junction sequence which consists of about 10 bases) which exists in the upstream region (enhancer field) of the various genes on genomic DNA, and activates the imprint of a gene. A NF-kappa B junction sequence exists also in the upstream region of genes, such as inflammatory cytokine, such as IL-1 besides an immunoglobulin gene, and a tumor necrosis factor, interferon, and a cell adhesion factor, and inflammation and an immune response require NF-kappa B as Seki through these gene expression induction.
[0004] NF-kappa B gets down also to symptoms formation of an autoimmune disease or an inflammatory disease as Seki, and it is known that the drug which has the activation depressant action of NF-kappa B shows effectiveness to the therapy and prevention of autoimmune diseases (rheumatoid arthritis, systemic lupus erythematosus, systemic scleroderma, Behcet's disease, a periarteritis nodosa, ulcerative colitis, glomerulonephritis, etc.), the intractable disease which presents an inflammation symptom, various viral diseases (the osteoarthritis, atherosclerosis, psoriasis, atopic dermatitis, etc.), the endotoxin shock, septicemia, etc. of a disease. And retrieval research of the activation depressant of NF-kappa B new for the therapy and prophylactic development of these diseases is advanced (Kopp et al., Science, the 265th volume, the 956th page, 1994; Baeuerle et al., the 65th volume of Advances in Immunology, the 111-137th page, 1997; JP,7-291859,A; JP,9-227561,A).
[0005] In retrieval research of the conventional NF-kappa B activation depressant, as the screening approach of a drug, or the identification approach, it cultivates under existence of a test drug or nonexistence under existence of a stimulus of a cell (or under nonexistence) by in vitro one, and, generally the method of detecting activation of NF-kappa B is used.
[0006] However, for a signal transfer path after receiving the stimulus (signal) with a fixed cell until it results in activation of NF-kappa B, existence of many steps with which various transfer molecules, such as protein kinase, are concerned can be considered. Therefore, for more efficient innovative drug development research, to establish the new drug screening approach of having focused on them after clarifying the transfer molecule which plays main roles is desired. However, the mechanism of activation of NF-kappa B some transfer factors (TRAF2 (TNF-alpha receptor

associated factor 2) —) NIK which is one of the MAPKKK(s) (NF-kappa B-inducing kinase), An IkappaB kinase (IKK), a ubiquitin conjugation enzyme, 26S pro TEOSOMU, etc. are identified, although solved little by little (it Nature(s) Nikolai et al. —) The 385th volume, the 540-544th page; Maniatis, Science, The 278th volume, the 818-819th page, 1997; Baeuerle et al., Advances in Immunology There were many still unknown points and the screening approach which focused on the elucidation of the mechanism which progressed more, and a new transfer molecule was desired in the 65th volume, the 111-137th page, and 1997.

[0007] On the other hand, the TGF-beta AKUCHIBETEDDO kinase 1 (it is also called Transforming growth factor-beta-activated kinase 1;TAK1) is found out as one of the MAPKKK(s) (mitogen-activated protein kinase kinase kinase) of mammalian (Yamaguchi et al., Science, the 270th volume, the 2008-2011st page, 1995; JP,9-163990,A). TAK1 activates PAI-1 promoter controlled by TGF-beta (transforming growth factor-beta). Moreover, since TGF-beta receives activation as it is also the origin of the naming, it has been thought that it is acting in the intracellular transfer path of the signal by the member of a TGF-beta super family.

[0008] Moreover, TAK1 serves as an activity form by combining with TAK1 binding protein 1 (it also being called TAK1 binding protein 1;TAB1) (interaction), and functioning as MAPKKK in a signal transfer path is known (Shibuya et al., Science, the 272nd volume, the 1179-1182nd page, 1996). However, about the relation of TAK1 and NF-kappa B activation, it was not known at all.

[0009]

[Problem(s) to be Solved by the Invention] The purpose of this invention is to offer the identification approach of NF-kappa B activation depressant and the screening approach of having focused on a new transfer molecule. Moreover, it is in offering the new identification approach and the screening approaches of a remedy and a prophylactic, such as an autoimmune disease and an intractable disease which presents an inflammation symptom.

[0010] Furthermore, it is in offering a remedy and prophylactics, such as new NF-kappa B activation depressant obtained by said approach and an autoimmune disease, and an intractable disease which presents an inflammation symptom.

[0011]

[Means for Solving the Problem] this invention persons found out that activation of NF-kappa B took place by isolating three allele variants (variant) of human TAK1cDNA, and carrying out manifestation enhancement (over expression) of Homo sapiens TAK1 with TAB1 in the research using these further. Moreover, interacting with IKK (IkappaB kinase) complex and participating in the activation, while TAK1 interacts with TAB1, and TAK1 of the variant which lost kinase activity further found out checking NF-kappa B activation.

[0012] From these knowledge, TAK1 is an important transfer molecule in the signal transfer path (NF-kappa B activating pathway) of resulting in activation of NF-kappa B, and it came to complete a header and this invention for the ability of the drug which controls the function of TAK1 to serve as activation depressant of NF-kappa B.

[0013] That is, this invention is the identification approach of NF-kappa B activation depressant or the screening approach including the process which authorizes a modulation operation of the examined substance to the function of TAK1 (TGF-beta AKUCHIBETEDDO kinase 1).

[0014] Moreover, they are the identification approach of the remedy of the intractable disease which presents an autoimmune disease or inflammation symptom including the process which authorizes a modulation operation of the examined substance to the function of TAK1 in NF-kappa B activating pathway, and/or a prophylactic, or the screening approach.

[0015] Furthermore, they are a remedy and prophylactics, such as new NF-kappa B activation depressant chosen or identified by said approach and an autoimmune disease, and an intractable disease which presents an inflammation symptom.

[0016]

[Embodiment of the Invention] TAK1 used in this invention may be the thing of which the seed origin, for example, the thing of the mammalian origins, such as Homo sapiens, a mouse, a rat, a rabbit, Buta, a dog, an ape, and a guinea pig, is mentioned. When using for researches and developments of a human remedy, it is [among these] desirable to use the thing of the Homo

sapiens origin.
[0017] The cDNA array and amino acid sequence of TAK1 are already reported (Genbank/EMBL database Accession No. D76446; Yamaguchi et al., Science, the 270th volume, the 2008-2011st page, 1995). Moreover, artificers showed the amino acid sequence of TAK1 by which a code is carried out to the DNA array of three allele variants (variant) of newly found-out human TAK1cDNA, and them to the array numbers 3, 4, and 5 of the after-mentioned array table.
[0018] According to the knowledge which artificers found out uniquely as aforementioned, TAK1 functions as main transfer molecules in NF-kappa B activating pathway.
[0019] It activates by carrying out an interaction (association) to TAB1 (TAK1 binding protein 1) by intracellular, and although TAK1 serves as an active type in which protein kinase activity (MAPKKK activity) is shown, it produces the formation of self-Lynn, and the phosphorylation of TAB1 by this interaction. Moreover, TAK1 interacts also with IKK complex functionally. Activated TAK1 activates IKK complex, demonstrates the function as a transfer molecule in NF-kappa B activating pathway, and is considered to guide NF-kappa B activation.
[0020] The mimetic diagram of the function of TAK1 in NF-kappa B activating pathway was shown in drawing 9 .
[0021] In this invention, an operation (especially inhibition or depressant action) of an examined substance is authorized paying attention to the above functions (function especially in the activating pathway of NF-kappa B) of TAK1. as such a function -- more -- concrete -- (1) -- the interaction (association) of TAK1 and TAB1, the protein kinase activity of (2) TAK1, activation of the IKK complex by intracellular TAK1, the NF-kappa B activation guided by (3) (4) intracellular TAK1, etc. are mentioned. [for example,] How to authorize an operation of the examined substance to these functions is described below.
[0022] (1) the approach of carrying out direct detection of assay, for example, the association with TAK1 and TAB1, of the operation over the interaction (association) of TAK1 and TAB1, and ********** (co-immunoprecipitation) -- the approach of detecting by law or approaches, such as a two hybrid system (two-hybrid system) (United States patent 5283173rd and Proc.Natl.Acad.Sci.USA, the 88th volume, the 9578 - 9582nd page, 1991), can be used.
[0023] Although those whole may be used as TAK1 and TAB1 in case association with TAK1 and TAB1 is detected, a partial polypeptide including the field which participates in both association at least may be used. Or the fusion protein which added the suitable tag indicators (partial polypeptides, such as a glutathione-S-transferase, 6xHis, protein A, the beta-galactosidase, a maltose-binding protein, a flag antigen, a Xpress antigen, a HA antigen, and a Myc antigen etc.) for them may be used.
[0024] When carrying out direct detection of the association with TAK1 and TAB1, association with the fusion protein which added the suitable tag indicator to TAB1 (or TAK1) if needed using TAK1 (or TAB1) which carried out the indicator by RI etc. is directly detected under existence of an examined substance.
[0025] ********** (co-immunoprecipitation) -- when based on law, the antibody which recognizes the tag indicator added to TAK1, TAB1, or these is used for detection. First, a cell solution is prepared from the cell which has discovered TAK1 and TAB1, and immunoprecipitation of the protein in a cell solution is carried out using the antibody which recognizes one protein. The interaction (association) of both intracellular protein is detectable by detecting existence of another [which is contained in the fraction which carried out immunoprecipitation] protein by approaches, such as immunity blotting.
[0026] Moreover, a two hybrid system is the approach of using the manifestation of a reporter gene as a marker (United States patent 5283173rd and Proc.Natl.Acad.Sci.USA, the 88th volume, the 9578 - 9582nd page, 1991).
[0027] The gene which carries out the code of the first fusion protein which specifically consists of the first field (a DNA binding field or imprint activation field) of the (i) transcription factor, and TAK1 when using a two hybrid system, (ii) The gene which carries out the code of the second fusion protein which consists of the second field (the imprint activation field or DNA binding field) and TAB1 of a transcription factor, And (iii) the reporter gene connected with the response array which

the DNA binding field of a transcription factor can combine, and its lower stream of a river, Using the cell for a ****** trial, this is made to coexist with an examined substance, it incubates, and an operation of the examined substance to association of TAK1 and TAB1 is authorized by making the manifestation of a reporter gene into an index. When an examined substance checks association of TAK1 and TAB1, reduction of reporter activity is accepted by existence of an examined substance.
[0028] The gene which carries out the code of the first and the second fusion protein can be designed and built using the usual gene modification technology.
[0029] As for a host cell, for example, a yeast cell, an insect cell, a mammalian cell, etc. are mentioned. When culture can be easy and it can carry out quickly, as for a yeast cell, it is [among these] advantageous at an easy point to apply gene modification technology, such as installation of a foreign gene.
[0030] what a transcription factor functions on by host intracellular — it is — ****ing — for example, GAL4 protein (Keegan et al. —) of yeast Science, the 231st volume, the 699 – 704th page, 1986, Ma et al., Cell, 48th volume, 847 – 853rd page, 1987, and GCN4 protein (Hope et al. —) ADR1 protein (Thukral et al., Molecular and Cellular Biology, the 9th volume, the 2360 – 2369th page, 1989) etc. will be mentioned in Cell, the 46th volume, the 885 – 894th page, and 1986.
[0031] GAL4 called UASg (about the upstream region activation section of a galactose metabolic turnover gene : upstream activation site of galactose genes) as a response array that a response array should just use the response array corresponding to a transcription factor when using GAL4 as a transcription factor — a specific DNA array can be used.
[0032] A reporter gene is not limited especially, either. For example, the gene of stable enzymes with easy quantitive measurement of activity, such as a beta-galactosidase gene (lacZ) of the Escherichia coli origin, a chloramphenicol acetyltransferase gene (CAT) of the bacteria transposon origin, and a luciferase gene (Luc) of the firefly origin, etc. can be used suitably.
[0033] (2) The solution which contains TAK1 and TAB1 in assay of the operation over the protein kinase activity of TAK1, for example, the solution containing a protein matrix, and the solution containing ATP (what carried out the indicator by RI etc. if needed) are added, perform an enzyme reaction under existence of an examined substance or nonexistence, measure protein kinase activity by making incorporation of the phosphoric acid to a protein matrix etc. into an index, and authorize an operation of an examined substance.
[0034] The thing made to discover with gene modification technology by suitable host cells (a yeast cell, an insect cell, mammalian cell, etc.) can be used for TAK1 and TAB1. Moreover, TAK1 in which the N terminal region of TAK1 was participating in association with TAB1, and the amino terminal (amino terminal side 22 amino acid) carried out deletion By that by which acting as an active signal transfer molecule is known also when not combining with TAB1 (Yamaguchi et al., Shibuya et al.) Instead of using both TAK1 and TAB1, the activity variant TAK1 which an amino terminal carries out deletion and shows activity in TAB1 un-depending may be used.
[0035] As a protein matrix, TAK1 the very thing, TAB1, or those partial peptides can be used. Moreover, the molecules which interact on IKK and IKK complex, and a functional target, or those partial peptides can also be used as a protein matrix.
[0036] XMEK2 [in addition,] (SEK1) (Shibuya et al. —) of a platanna Science, the 272nd volume, the 1179-1182nd page, 1996, Homo sapiens MKK3 (Derijard [et al.] and Science — the 267th volume) the 682-685th page, 1995, and Homo sapiens MKK6 (MAPKK6) (Raingeaud et al. —) Molecular and Cellular Biology, The 16th volume, the 1247-1255th page, 1996; Moriguchi et al., Journal of Biological Chemistry, MAPKK(s) (mitogen activated protein kinase kinase) and those partial peptides in the 271st volume, the 13675-13679th page, 1996, etc. can also be used as a substrate. When using MAPKK as a substrate, the protein kinase activity of TAK1 can also be measured by making activation (increase of the phosphorylation activity over MAPK (mitogen activated protein kinase)) of MAPKK into an index.
[0037] (3) Use assay of the operation over the IKK complex activation by intracellular TAK1, for example, the cell to which manifestation enhancement (over expression) of TAK1 (a detail TAK1 of an active type) was carried out, as a cell for a trial. As [both] such a cell for a trial, the cell which carried out manifestation enhancement of TAK1 and TAB1 is mentioned, and it is obtained by

introducing the vector for a manifestation of TAK1 and TAB1 into a suitable host cell. Or the cell which carried out manifestation enhancement of the activity variant TAK1 which an amino terminal carries out deletion and shows activity in TAB1 un-depending may be used.
[0038] Said cell for a trial is cultivated under existence of an examined substance or nonexistence. The fraction containing IKK complex is acquired from the cell after culture by immunoprecipitation etc., an IKK kinase reaction is performed using this, activation of IKK complex is measured, and an operation of an examined substance is authorized.
[0039] (4) Cultivate this under existence of an examined substance or nonexistence like assay (3) of the operation over the NF-kappa B activation guided by intracellular TAK1, for example, the above, using the manifestation enhancement cell of an active type TAK1 as a cell for a trial. Gel shift assay etc. detects NF-kappa B activation, and an operation of an examined substance is authorized.
[0040] The amount of manifestations of TAK1 which works as a signal transfer molecule as compared with control cells (cell which introduced only the vector) is increasing the manifestation enhancement cell of an active type TAK1. Therefore, it is suitable as a trial cell in the case of wanting to choose the test drug which acts on TAK1. For example, in both cell which carried out manifestation enhancement of the active type TAK1, and control cell, when NF-kappa B activation depressant action is accepted by existence of an examined substance, it is judged that the point of application of this examined substance has high possibility of being in TAK1.
[0041] The above (1) In the approach of - (4), as a cell used for a trial, the cell strain of the mammalian origins, such as Homo sapiens, can be used suitably, a Homo sapiens HeLa cell, a Homo sapiens Jurkat cell, Homo sapiens THP-1 cell, ape COS-7 cell, a Chinese hamster CHO cell, etc. are mentioned, among these a Homo sapiens HeLa cell, a Homo sapiens Jurkat cell, Homo sapiens THP-1 cell, etc. are desirable.
[0042] The above (1) In the approach of - (4), when carrying out manifestation enhancement of TAK1, TAB1, or these fusion proteins, it can carry out using known array information and the usual gene modification technology.
[0043] The array information on TAK1 is as aforementioned, and the cDNA array and amino acid sequence of TAB1 are also reported (Genbank/EMBL database Accession No.U49928; Shibuya et al., Science, the 272nd volume, the 1179-1182nd page, 1996). TAB1 may be the thing of which the seed origin, for example, the thing of the mammalian origins, such as Homo sapiens, a mouse, a rat, a rabbit, Buta, a dog, an ape, and a guinea pig, is mentioned. When using for researches and developments of a human remedy, it is [among these] desirable to use the thing of the Homo sapiens origin.
[0044] the primer and probe which designed cDNA or genes, such as TAK1 and TAB1, based on a known amino acid sequence, the information on a base sequence, etc., and were compounded -- using -- usual PCR (Polymerase Chain Reaction) -- it can isolate by screening from law, RT-PCR method, or a DNA library. These are included in a suitable vector and the vector for a manifestation can be built.
[0045] The vectors for animal cells (for example, a retrovirus system vector, a papillomavirus vector, a vaccinia virus vector, an SV40 system vector, etc.) which contain suitable promotors (for example, a CMV promotor, an SV40 promotor, an LTR promotor, an elongation 1alpha promotor, etc.) as a vector can be used.
[0046] The above (1) What is necessary is just to check the depressant action to NF-kappa B activation further about the examined substance the inhibitory action to a function and depressant action of TAK1 were accepted to be by the assay approach like - (4). Or what is necessary is just to check a therapy and/or a preventive effect in the known symptoms model (inch vitro or in vivo) of the intractable disease which presents an autoimmune disease or an inflammation symptom.
[0047] NF-kappa B activation -- the known gel shift assay method (Sakurai et al. --) Journal ofNeurochemistry The 59th volume, the 2067-2075th page, 1992; Sakurai et al., Biochimica Biophysica Acta, the 1316th volume, the 132-138th page, 1996, and the reporter assay method (Tanaka et al. --) Journal of Veterinary Medical Science, the 59th volume, the 575 - 579th page, 1997;EP-652290-A; JP,7-291859,A; JP,9-227561,A etc. can investigate.
[0048] As a known symptoms model (inch vitro or in vivo) of the intractable disease which presents

an autoimmune disease or an inflammation symptom the PHA induction IL-2 production model (Wacholtz et al. —) using a Homo sapiens T cell stock (Jurkat cell) Cell Immunology, the 135th volume, the 285 - 298th page, the LPS+IFN-gamma induction iNOs production model (Xie et al. —) using 1991 and the Homo sapiens macrophage system cell RAW 264.7 in vitro models, such as a TNF-alpha induction IL-6 production model using Science, the 256th volume, the 225 - 228th page, 1992, and a Homo sapiens HeLa cell, a rat adjuvant arthritis model (European Journal of Pharmacology Connor et al. —) the 273rd volume, the 15 - 24th page, 1995, and a trinitro benzenesulfonic acid induction colitis model (Kiss et al. —) European Journal of Pharmacology, in vivo models, such as the 336th volume, the 219 - 224th page, 1997, and a rat Masugi's nephritis model (Sakurai et al., Biochimica BiophysicaActa, the 1316th volume, the 132-138th page, 1996), etc. are mentioned.

[0049] Hereafter, although this invention is explained in more detail with an example, these examples do not restrict this invention.

[0050] In addition, in the following example, as long as there was no designation, especially each actuation was used according to the instructions of a commercial item, when it carried out to "molecular cloning (Molecular Cloning)" (Sambrook, J., Fritsch, E.F. and Maniatis, and T. it will be published from work and Cold Spring Harbor Laboratory Press in 1989) by the approach of a publication or a commercial reagent and a commercial kit were used.

[0051]

[Example] Example 1 cDNA isolation of Homo sapiens TAK1 and TAB1 (1) Pori (A) RNA was prepared from Homo sapiens's TAK1 cDNA isolation Homo sapiens uterus ****** origin cell strain HeLa (ATCC CCL2). This was made into mold and the single strand cDNA was prepared using the oligo dT primer.

[0052] the single strand cDNA obtained above — mold — carrying out — PCR (polymerase chain reaction) — Homo sapiens's TAK1 cDNA fragment was acquired by law. The primer used for PCR referred to the cDNA array (Genbank/EMBL database Accession No. D76446; Yamaguchi et al., Science, the 270th volume, the 2008-2011st page, 1995) of a mouse TAK1, designed it, and compounded it with the DNA synthesis machine. The synthetic primer (array number 1 of the after-mentioned array table) of 30 MA which consists of an array (ten bases) which includes the recognition site for restriction enzyme cutting as a sense primer, and the translation initiation codon and the array of a lower stream of a river of mouse TAK1cDNA (20 bases) is used. The synthetic primer (array number 2 of the after-mentioned array table) of 30 MA which consists of an array (ten bases) which includes the recognition site for restriction enzyme cutting as an antisense primer, and a complementary array (20 bases) of the termination codon and the upstream of mouse TAK1cDNA was used.

[0053] cDNA (hTAK1 a-cDNA and hTAK1 b-cDNA) including all two sorts of Homo sapiens' TAK1 coding regions was acquired by using as a probe the product (mixture of the cDNA fragment of about 1.7 kbs) acquired by said PCR, and screening a Homo sapiens lung cDNA library (product made from Clontech).

[0054] moreover, mRNA of HeLa prepared like the above — mold — carrying out — RT-PCR (Reverse transcript-polymerase chain reaction) — cDNA (hTAK1 c-cDNA) including all Homo sapiens's TAK1 coding regions was separately obtained by law. As a primer, the same synthetic primer as the above was used.

[0055] About three sorts of obtained cDNA(s), the DNA array was determined by the dideoxy method. Homo sapiens's TAK1 (hTAK1a, hTAK1b, and hTAK1c) amino acid sequence by which a code is carried out to the DNA array of a field and them including the coding region about each cDNA (hTAK1 a-cDNA, hTAK1 b-cDNA, and hTAK1 c-cDNA) was shown in the array number 3, the array number 4, and the array number 5 of the after-mentioned array table.

[0056] Homology [in / as compared with the cDNA array of a mouse TAK1 / in the cDNA array of hTAK1a, hTAK1b, and hTAK1c / a coding region] was 91.7%, 87.6%, and 86.8% respectively.

[0057] hTAK1a consists of 579 amino acid residue. The permutation of 4 amino acid was seen as compared with the mouse TAK1, and the homology in an amino acid sequence was 99.3%.

[0058] hTAK1b consists of 606 amino acid residue, and insertion of 27 amino acid considered to

have been generated by splicing variation in the C terminal side as compared with hTAK1a is seen. Moreover, hTAK1c consists of 567 amino acid residue, a C terminal has insertion of 27 amino acid like hTAK1b as compared with hTAK1a, and the deletion of 39 amino acid was seen further on the lower stream of a river (C terminal side).
[0059] The comparison of the amino acid sequence of three sorts of Homo sapiens TAK1 and a mouse TAK1 was shown in drawing 1 .
[0060] In addition, as compared with the amino acid sequence of hTAK1a, the permutation (372nd Arg->His) of 1 amino acid is seen, and TAK1 of the Homo sapiens T cell stock Jurkat origin indicated by the array number 5 of JP,9-163990,A is considered to be an allele variant.
[0061] (2) Pori (A) RNA prepared from HeLa like Homo sapiens's TAB 1 cDNA isolation preceding clause (1) was used as mold, and Homo sapiens's TAB 1 cDNA was obtained by RT-PCR. The primer referred to Homo sapiens's TAB 1 cDNA array (Genbank/EMBL database Accession No.U49928; Shibuya et al., Science, the 272nd volume, the 1179-1182nd page, 1996) reported, designed it, and compounded it with the DNA synthesis machine. The synthetic primer (array number 6 of the after-mentioned array table) of 30 MA which consists of an array (ten bases) which includes the recognition site for restriction enzyme cutting as a sense primer, and the translation initiation codon and the array of a lower stream of a river of TAB1cDNA (20 bases) is used. As an antisense primer The synthetic primer (array number 7 of the after-mentioned array table) of 30 MA which consists of an array (ten bases) including the recognition site for restriction enzyme cutting and a complementary array (20 bases) of the termination codon and the upstream of TAB1cDNA was used.
[0062] The DNA array was determined about the obtained cDNA fragment, and it checked including all known Homo sapiens's TAB 1 coding regions.
[0063] Example 2 Detection of the NF-kappa B activation in the cell which reinforced the manifestation of TAK1 (1) Three sorts of Homo sapiens TAK1cDNA(s) acquired in (1) of the acquisition aforementioned example 1 of a cell which reinforced Homo sapiens's TAK1 manifestation are used. a partial fragment (the EcoRI-NheI fragment of hTAK1 a-cDNA --) including the coding region Each of the EcoRI-NheI fragment of hTAK1 b-cDNA, and the EcoRI-XbaI fragment of hTAK1 c-cDNA It included at least in the EcoRI-XbaI cutting section of the vector plasmid pcDNA3.1 for an eukaryotic cell manifestation (+), and a (the product made from Invitrogen), and the recombination plasmid for TAK1 manifestation was produced.
[0064] Moreover, the partial fragment (HindIII-EcoRI fragment) including the coding region was built at least into the HindIII-EcoRI cutting section of the vector plasmid pcDNA3.1 for a manifestation (+) using Homo sapiens TAB1cDNA acquired by (2) of said example 1, and the recombination plasmid for TAB1 manifestation was produced.
[0065] said recombination plasmid for TAK1 manifestation -- the recombination plasmid for TAB1 manifestation -- or transfection (transient transfection; transient transfection) was independently carried out to the HeLa cell. At this time, transfection was performed using the cationic liposome for transfection (trade name: LipofectAMINE, product made from Life Technologies).
[0066] The TAK1 manifestation enhancement cell or the TAK1-TAB1 coexpression enhancement cell was obtained in this way. Culture of these cells was performed in the high glucose content Dulbecco-Eagle's medium (product made from Gibco) which added fetal calf serum, penicillin (100 units / ml), and streptomycin (100microg/(ml)) 10%.
[0067] (2) According to the approach given in reference (Sakurai et al., the 59th volume of Journal of Neurochemistry, the 2067-2075th page, 1992; Sakurai et al., Biochim.Biophys.Acta, the 1316th volume, the 132-138th page, 1996), gel shift assay was performed as follows using the TAK1 manifestation enhancement cell and TAK1-TAB1 coexpression enhancement cell which were obtained for the gel shift assay preceding clause (1). That is, after transfection, the cell was cultivated and the nucleus extract was prepared from the cell 24 hours after.
[0068] After carrying out the ligation reaction of the probe for detection which carried out RI indicator to this nucleus extract (5microg) for 30 minutes at a room temperature among the joint buffer solution (20mMHEPES (pH7.9), 0.3mM EDTA, 0.2mM EGTA, 80mM NaCl, and 10% glycerol and 2microg/ml poly [dI-dC]), polyacrylamide gel electrophoresis was performed about reaction mixture.

After carrying out reduced pressure drying of the gel, NF-kappa B combined with the probe with autoradiography was detected. Moreover, Oct-1 (Octamer-1) (Verrijzer et al., Genes and Development, the 4th volume, the 1964 - 1974th page, 1990) which is the transcription factor discovered in configuration as control was detected.

[0069] The synthetic DNA of the double strand which carried out the indicator by 32P was used for the probe for detection. The thing same as an array of the probe for NF-kappa B detection as the NF-kappa B junction sequence which exists in LTR (Long Terminal Repeat) of HIV was used. Moreover, the oligonucleotide containing consensus sequence AGCTAAAT was used as an array of the probe for Oct-1 detection.

[0070] As it was the above, when manifestation enhancement of Homo sapiens TAK1 (hTAK1a, hTAK1b, or hTAK1c) was carried out with TAB1 as it was shown in drawing 2 , as a result of gel shift assay's investigating NF-kappa B activation by making nuclear shift of NF-kappa B into an index, the shift to the nucleus of NF-kappa B was seen, and activation of NF-kappa B was accepted. Although such a result was accepted as Homo sapiens TAK1 also when any of hTAK1a, hTAK1b, and hTAK1c were used, activation of NF-kappa B was remarkable in especially hTAK1b.

[0071] On the other hand, activation of NF-kappa B was not accepted in the cell which carried out manifestation enhancement only of Homo sapiens TAK1. Moreover, Oct-1 detected as control protein did not receive effect in manifestation enhancement of TAK1 and/or TAB1, but the manifestation was seen constantly.

[0072] Thus, since activation of NF-kappa B was observed with enhancement of an operation of Homo sapiens TAK1, in the signal transfer path until TAK1 results in activation of NF-kappa B, it turned out that work main as a transfer molecule is carried out.

[0073] (3) Reporter assay (luciferase assay)

According to the approach given [Tanaka and others] in reference (Tanaka et al., Journal of Veterinary Medical Science, the 59th volume, the 575 - 579th page, 1997), as it was the following, reporter assay (luciferase assay) was performed.

[0074] First, NF-kappaB Junction sequence (GGGGACTTTCC) Oligonucleotide connected four pieces Firefly luciferase gene (Luc) It included in the upstream and the reporter plasmid (p(kB)4-Luc) was produced.

[0075] Next, according to the approach given in the preceding clause (1), transfection (transient transfection; transient transfection) of the recombination plasmid for TAK1 manifestation was carried out to the HeLa cell with the recombination plasmid for TAB1 manifestation if needed. On the occasion of **** transfection, both the reporter plasmids (p(kB)4-Luc) obtained above were used.

[0076] Transformer FEKUTANTO which contains a reporter plasmid and the recombination plasmid for TAK1 manifestation (and recombination plasmid for TAB1 manifestation) in this way was obtained. After cultivating obtained transformer FEKUTANTO for 48 hours, luciferase activity was measured about the extract which dissolved and prepared the cell. Luciferase activity was measured using the luciferase assay kit, the picker gene (a trade name, Toyo Ink make), and the chemiluminescence measuring device (MicroLumant LB96P, made in Berthold Japan, Inc.).

[0077] Consequently, in the cell which carried out manifestation enhancement only of Homo sapiens TAK1 (hTAK1a, hTAK1b, or hTAK1c), most increments in luciferase activity (namely, activation of NF-kappa B) were not accepted as compared with the cell only containing a vector as shown in drawing 3 . However, in the cell which carried out manifestation enhancement of Homo sapiens TAK1 with TAB1, the remarkable increment in luciferase activity (namely, activation of NF-kappa B) was accepted as compared with the cell only containing a vector.

[0078] Thus, like the aforementioned gel shift assay method, activation of NF-kappa B was observed with enhancement of an operation of Homo sapiens TAK1 also by the reporter assay method (the luciferase assay method), and it was checked that TAK1 is carrying out work main as a transfer molecule.

[0079] Moreover, it is thought that the operation over the operation and NF-kappa B activation of a test drug to TAK1 can be authorized to coincidence in this way by the system of the reporter assay using a TAK1 manifestation enhancement cell and a control cell.

[0080] Example 3 The translation field of Homo sapiens TAK1cDNA obtained by (1) of the joint detection system aforementioned example 1 of TAK1 and TAB1 using a two hybrid system is started, and it inserts in the multi-cloning part of the expression vector pGBT9 (the product made from Clontech, vector for yeast two-hybrid systems) containing DNA which carries out the code of the DNA binding field (1 to 147th amino acid residue of GAL4) of a transcription factor GAL4 for this. This obtains plasmid pGBT9-TAK1 for discovering the fusion protein of the DNA binding field of GAL4, and Homo sapiens TAK1.
[0081] The translation field of Homo sapiens TABcDNA who got by (2) of said example 1 is started, and it inserts in the multi-cloning part of the expression vector pGAD424 (the product made from Clontech, vector for yeast two-hybrid systems) containing DNA which carries out the code of the imprint activation field (768 to 881st amino acid residue of GAL4) of GAL4 for this. This obtains plasmid pGAD424-TAB1 for discovering the fusion protein of the imprint activation part of GAL4, and TAB1.
[0082] Fusion protein manifestation plasmid pGBT9-TAK1 and pGAD424-TAB1 which are obtained above are introduced into the host yeast cell strain SFY526 (product made from Clontech). A cell strain SFY526 is a cell strain which the fusion gene of GAL1 and lacZ is included in the chromosome, and has the deficit variation of GAL4 gene (Bartel et al., Bio Techniques, the 14th volume, the 920 - 924th page, 1993). A transformation sorts out by cultivating in the synthetic medium made to lack the tryptophan and leucine which are the selective marker of each plasmid, and obtains the transformant into which both plasmids were introduced.
[0083] The yeast transformant obtained above is cultivated by the liquid medium. In a culture medium, an examined substance is added in the case of culture (or additive-free). Centrifugal separation recovers a yeast-fungus object after 4 - 5-hour culture, and association (interaction) of TAK1 and TAB1 is detected by making beta-galactosidase activity into an index.
[0084] When a concentration dependence target is permitted reduction of beta-galactosidase activity by addition of an examined substance, by it, to have the operation which checks association of TAK1 and TAB1 is considered by the examined substance.
[0085] Example 4 It is made discovered by the system of an insect cell, and detection system Homo sapiens TAK1 (or Homo sapiens TAK1 as for whom the amino terminal (22 amino acid) did deletion) of the MAPKKK activity of TAK1 is refined, as it is the following. That is, it inserts in the multi-cloning part of Baculoviridae manifestation vector pAcHLT including the suitable DNA array designed in order to add a tag peptide (6xHis or glutathione-S-transferase), or pAcGHLT (product made from fur MINJIEN) using the translation field of Homo sapiens TAK1cDNA obtained by (1) of said example 1, and a Homo sapiens TAK1 manifestation plasmid is obtained. Cultivate the transformed cell which might be introduced into the host insect cell SF 21 in the obtained plasmid, Homo sapiens TAK1 (or amino terminal deletion Homo sapiens TAK1) to whom the tag peptide was added is made to discover, and the affinity chromatography which uses the added tag peptide from a cell extract refines.
[0086] Moreover, like the above, it is made discovered by the system of an insect cell, and Homo sapiens TAB 1 is refined.
[0087] Moreover, it is the following, and it is made discovered [make and] and Homo sapiens MKK3 and Homo sapiens MKK6 are refined. First, MORIGUCHI's and others (Moriguchi) approach (Journal of Biological Chemistry) the array information (Genbank/EMBL database Accession No.L36719; — Derijard et al: —) concerning Homo sapiens MKK3 according to the 271st volume, the 13675-13679th page, and 1996 the array information (Genbank/EMBL database Accession No.U39656 and U39657; — Raingeaud et al. —) about Science, the 267th volume, the 682-685th page, 1995, and Homo sapiens MKK6 Will design a primer based on Molecularand Cellular Biology, the 16th volume, the 1247-1255th page, and 1996, and by the PCR method using these cDNA including all the translation fields of Homo sapiens MKK3 and Homo sapiens MKK6 or cDNA including the array near the amino acid residue in which phosphorylation is carried out by TAK1 is acquired. It inserts in the multi-cloning part including the suitable DNA array designed in order to add a tag peptide (6xHis or glutathione-S-transferase) of Escherichia coli expression vector pQE-30 (product made from QIAGEN), or pGEX-2T (Pharmacia manufacture) using these cDNA(s), and a Homo

sapiens MKK3 manifestation plasmid and a Homo sapiens MKK6 manifestation plasmid are obtained. Cultivate the transformed cell which might be introduced into host Escherichia coli (109 shares of JM etc.) in the plasmid obtained, Homo sapiens MKK3 and Homo sapiens MKK6 to whom the tag peptide was added are made to discover respectively, and the affinity chromatography which uses the added tag peptide from a cell extract refines.

[0088] Homo sapiens TAK1 (or amino terminal deletion Homo sapiens TAK1) obtained above is used as an enzyme (MAPKKK) combining Homo sapiens TAB 1 if needed, and an enzyme reaction is performed under existence of an examined substance or nonexistence, using Homo sapiens MKK3 or Homo sapiens MKK6 as a substrate. Beforehand, a protein matrix is solid-phase-ized, and is used on a plate, and a reaction is performed at 30 degrees C among the tris buffers (20 mM Tris-HCl, pH 7.5, 2mM EGTA, 10mM MgCl2) containing 32P or 33P indicator ATP100microM. After an enzyme reaction, after washing a plate, by measuring and carrying out incorporation of the 32P or 33P indicator ATP with a scintillation counter, enzyme activity is measured and the existence of inhibition by the examined substance is judged.

[0089] Example 5 As it was below control of the NF-kappa B activation in the cell which made the variant TAK1 discover, the existence of NF-kappa B activation was detected using the cell which carried out manifestation enhancement of the variant TAK1 (or wild type TAK1) lacking in kinase activity.

[0090] (1) Expression vector construction of TAK1 and TAB1 and transfection vector plasmid pFLAG-CMV2 are the vectors for making the protein which added the tag of a flag antigen discover in a mammalian cell. The expression vector of the wild type TAK1 (Flag-TAK1) by which flag addition was carried out was obtained by including Homo sapiens's TAK1 (Homo sapiens TAK1a) overall length cDNA at least in the EcoRI-XbaI restriction enzyme cutting section of pFLAG-CMV2 (product made from Kodak).

[0091] Moreover, using the kit for variation installation (product made from QuickChange site-directed mutagenesis kit;Stratagene), variation installation was carried out to the TAK1 translation field of said Flag-TAK1 expression vector, various variation expression vectors were acquired, and the base sequence was determined. The expression vector of the variant TAK1 (Flag-TAK1K63W) by which flag addition was carried out in this way was obtained. The 63rd lysine residue of a wild type TAK1 is permuted by tryptophan residue, and the variant TAK1 discovered by this expression vector had lost the kinase activity of TAK1.

[0092] Transfection of the expression vector of the aforementioned wild type by which flag addition was carried out, or a variant TAK1 (Flag-TAK [1] or Flag-TAK1K63W) was carried out to the HeLa cell with independent or TAB1 expression vector, and it was made to discover transient. Moreover, as control, it changed to the TAK1 expression vector and only the vector was used. Performing transfection using the lipofectamine reagent (product made from Life Technologies), the expression vector of TAB1 used the same thing as said example 2 (1).

[0093] (2) Gel shift assay was performed like the example 2 (2) using the cell which was obtained with the gel shift assay above (1) and which carried out manifestation enhancement of the variant TAK1 (or wild type TAK1) by which flag addition was carried out with TAB1.

[0094] Consequently, as compared with the cell which introduced only the vector, in the cell which carried out manifestation enhancement of the wild type TAK1 (Flag-TAK1) with TAB1, nuclear shift of NF-kappa B was reinforced and NF-kappa B activation was accepted as shown in (A) of drawing 4 . However, even if it made it discovered with TAB1 in the case of the variant TAK1 (Flag-TAK1K63W) lacking in kinase activity, nuclear shift of NF-kappa B was not reinforced.

[0095] (3) Reporter assay (luciferase assay)

Transfection of the expression vector of a variant TAK1 (Flag-TAK1K63W) obtained above (1) was carried out to the HeLa cell. the amount of the Flag-TAK1K63W expression vector used for **** transfection — three kinds, 0microg, 0.03microg, and 0.1microg, — carrying out — the total amount of DNA — being the same (0.1microg) — it adjusted by the vector plasmid so that it might become.

[0096] Moreover, on the occasion of transfection, transfection of the reporter plasmid (p(kB)4-Luc containing a NF-kappa B junction sequence and a firefly luciferase gene) obtained by (3) of an example 2 was added and carried out to coincidence.

[0097] 24 hours after transfection and in the culture medium, TNF-alpha was added so that it might become the 20 ng/ml last concentration (control was made TNF-alpha additive-free). Furthermore, like (3) of an example 2 after 5-hour culture, the cell was dissolved and luciferase activity was measured.
[0098] The result was shown in drawing 4 (B) (the-less mark of TAK1K63W, +, and ++ express respectively addition 0microg, 0.03microg, and 0.1microg of Flag-TAK1K63W expression vector among drawing.). The increment in the luciferase activity guided by the TNF-alpha stimulus (activation of NF-kappa B) was controlled depending on the dosage of the variant TAK1 expression vector used for transfection as shown in drawing 4 (B).
[0099] From this result, by making the variant TAK1 lacking in kinase activity discover by intracellular showed controlling activation of NF-kappa B.
[0100] While this proves that it is the molecule with which TAK1 carries out main work in NF-kappa B activating pathway like the result of the above (2), the drug which checks the kinase activity of TAK1 and activation of TAK1 proves strongly that activation of NF-kappa B is controlled.
[0101] Example 6 As it was below the interaction of TAK1 and TAB1 in intracellular, the interaction (association) of TAK1 and TAB1 in intracellular was detected with the immunoprecipitation method using the cell which carried out manifestation enhancement of TAK1 with TAB1.
[0102] (1) Transfection of the expression vector of the wild type TAK1 (Flag-TAK1) by which flag addition was carried out, or a variant TAK1 (Flag-TAK1K63W) was carried out to the HeLa cell with independent or TAB1 expression vector like transfection **** of a cell, and an example 5.
[0103] (2) Cells were collected 24 hours after immunoprecipitation and immunity blotting transfection, and as it was the following, the cell solution (cell lysate) was prepared. That is, the cell was diluted 3 times, after dissolving using the lysis buffer solution (25mM HEPES (pH7.7), 0.3M NaCl, 1.5mM MgCl2, 0.2mM EDTA, 0.1% Triton X-100, 20mM beta-glycerophosphate, 0.1 mM sodium orthovanadate, 0.5mM PMSF, 1mM DTT, 10microg/ml aprotinin, 10microg/ml leupeptine), and it ice-cooled for 10 minutes. Supernatant liquid was isolated preparatively after centrifugal and it used for the following actuation by making this into a cell solution.
[0104] Ice-cooling incubation of the cell solution obtained above is carried out with an anti-flag antibody (M5, product made from KODAKKU) for 1.5 hours, protein G sepharose (product made from Pharmacia) is added further, it mixed gently for 1.5 hours and 4 degrees C of immune complexes were made to stick to a protein G sepharose bead. After centrifugal recovered this bead, the buffer solution for washing (20mM HEPES (pH7.7), 50mM NaCl, 2.5mM MgCl2, 0.1mM EDTA, 0.05% Triton X-100) washed 5 times, and it used for the following actuation by making this into an immunoprecipitation fraction.
[0105] After presenting SDS-polyacrylamide gel electrophoresis with said bead (immunoprecipitation fraction), it imprinted on the PVDF (polyvinylidene difluoride) film, immunity blotting was performed, and TAB1 and TAK1 which exist in an immunoprecipitation fraction were detected. As an antibody for detecting TAK1 and TAB1, anti-TAK1 antibody (M-17) (product made from Santa Cruz Biothechnology) and anti--TAB1 antibody (N-19) (product made from Santa Cruz Biothechnology) were used respectively.
[0106] The result of immunity blotting of an anti-flag immunoprecipitation fraction was shown in drawing 5 . It is an upper case as a result of [in anti-TAB1 antibody] detection, and the lower berth is as a result of [in anti-TAK1 antibody] detection.
[0107] TAB1 lived together in the anti-flag immunoprecipitation fraction of the cell which carried out manifestation enhancement of the wild type TAK1 (Flag-TAK1) as shown in drawing 5 . Moreover, in the cell which it changed [cell] to the wild type and carried out manifestation enhancement of the variant TAK1 (Flag-TAK1K63W), TAB1 lived together in the immunoprecipitation fraction similarly.
[0108] Thus, since TAB1 was **********(ed) with TAK1 (a wild type and variant), it turns out that TAK1 and TAB1 are interacting by intracellular.
[0109] Moreover, when coexpressing wild types TAK1 and TAB1, although the inclination for the mobility in SDS-polyacrylamide gel electrophoresis to decrease a little was seen for both, in the case of the variant TAK1 which does not have kinase activity, reduction of such mobility was not seen. Reduction of such mobility was considered to reflect that both protein received

phosphorylation by the functional interaction.
[0110] (3) Obtain the cell which carried out manifestation enhancement of TAK1 with TAB1 like the assay above (1) of an operation of an examined substance, and cultivate this under existence of an examined substance or nonexistence. About the cell after culture, the interaction (association) of TAK1 and TAB1 is detected with an immunoprecipitation method like the above (2). An operation of the examined substance to the interaction (association) of TAK1 and TAB1 of the examined substance is authorized by judging whether ********** of TAK1 and TAB1 decreases by existence of an examined substance.
[0111] Example 7 As it was the self-phosphorylation and 1 or less phosphorylation of TAB by TAK1, about TAK1 which carried out immunoprecipitation from the cell which carried out manifestation enhancement of TAK1 with TAB1, kinase assay was carried out and the self-phosphorylation by TAK1 and the phosphorylation of TAB1 were detected.
[0112] (1) Transfection of the expression vector of the wild type TAK1 (Flag-TAK1) by which flag addition was carried out, or a variant TAK1 (Flag-TAK1K63W) was carried out to the HeLa cell with independent or TAB1 expression vector like the transfection of a cell and immunoprecipitation ****, and an example 5. From the cell 24 hours after transfection, the cell solution was prepared like the example 6, and immunoprecipitation by the anti-flag antibody was performed.
[0113] (2) Using the anti-flag immunoprecipitation fraction obtained with the kinase assay above, as it was the following, the in vitro kinase reaction was performed.
[0114] that is, the immunoprecipitation fraction was added to the kinase buffer solution (20mM HEPES (pH7.6), 20mM MgCl2, 2mM DTT, 20microMATP, 20 mM beta-glycerophosphate, 20mM disodium p-nitrophenylphosphate, 0.1 mM sodium orthovanadate, and 3microcurie [gamma-32P] -- ATP) of 30microl, and 30 degrees C incubated for 30 minutes. SDS-polyacrylamide gel electrophoresis was presented with reaction mixture after reaction termination, and autoradiography was carried out about the gel after migration.
[0115] Consequently, in the anti-flag immunoprecipitation fraction of the cell which carried out manifestation enhancement of both of wild types TAK1 (Flag-TAK1) and TAB1, the phosphorylation (self-phosphorylation) of TAK1 and the phosphorylation of TAB1 were accepted as shown in drawing 6 . However, in the immunoprecipitation fraction of the cell which carried out manifestation enhancement only of the wild type TAK1, neither of the phosphorylation, TAK1 nor TAB1, was accepted. Moreover, about the variant TAK1 lacking in kinase activity, even when manifestation enhancement was carried out with TAB1, phosphorylation was not accepted.
[0116] From these things, TAK1 was activated by coexisting with TAB1, and it was thought that the self-phosphorylation of TAK1 and the phosphorylation of TAB1 by TAK1 happened.
[0117] Example 8 As it was below the interaction of TAK1 and IKK in intracellular, the interaction (association) of TAK1 and IKK in intracellular was detected with the immunoprecipitation method using the cell which carried out manifestation enhancement of TAK1 with IKK.
[0118] (1) every of transfection **** of a cell, Homo sapiens IKKalpha, and Homo sapiens IKKbeta -- the expression vector of IKK was acquired by including cDNA in the vector plasmid (+) (product made from Invitrogen) pcDNA3.1 HisB. What was acquired from mRNA of a Homo sapiens monocyte origin cell strain (THP-1) by reverse transcription PCR (Reverse transcriptase-polymerase chain reaction) was used for cDNA of Homo sapiens IKKalpha (Genbank/EMBL accessionNo.AF012890;Cell, the 90th volume, the 373 - 383rd page, 1997), and Homo sapiens IKKbeta (Genbank/EMBL accession No.AF029684;Science, the 278th volume, the 866 - 869th page, 1997).
[0119] IKK (Xpress-IKKalpha or Xpress-IKKbeta) to which the Xpress tag polypeptide was added can be made to discover by these IKK(s) expression vector (an IKKalpha expression vector and IKKbeta expression vector).
[0120] Next, transfection of the expression vector of the wild type TAK1 (Flag-TAK1) which carried out flag addition was carried out to the HeLa cell with independent or TAB1 expression vector like the example 5. Under the present circumstances, transfection also of the expression vector of IKK (Xpress-IKKalpha or Xpress-IKKbeta) obtained above was added and (or un-adding) carried out to coincidence.

[0121] (2) From the cell 24 hours after immunoprecipitation and immunity blotting transfection, immunoprecipitation according a cell solution to preparation and an anti-flag antibody was performed like the example 6. After performing SDS-polyacrylamide electrophoresis about an immunoprecipitation fraction and a cell solution, immunity blotting was performed and IKK and TAK1 were detected.
[0122] As an antibody for detecting IKK (Xpress-IKK alpha and beta) and TAK1, the anti-Xpress antibody (M-21) (product made from Santa Cruz Biothechnology) and anti--TAK1 antibody (M-17) (product made from Santa Cruz Biothechnology) were used respectively.
[0123] The result of immunity blotting of an anti-flag immunoprecipitation fraction was shown in drawing 7 .
[0124] The detection result according [the detection result according / an upper case / to the anti-Xpress antibody of an anti-flag immunoprecipitation fraction and the middle] to the anti-Xpress antibody of a cell solution and the lower berth are as a result of [by anti-TAK1 antibody of an anti-flag immunoprecipitation fraction] detection.
[0125] Manifestation enhancement of TAK1 (Flag-TAK1) and the IKK (Xpress-IKKalpha or Xpress-IKKbeta) was carried out, and IKK was detected in the anti-flag immunoprecipitation fraction in the cell in which TAB1 did not carry out manifestation enhancement as shown in drawing 7 . Thus, since IKK (IKK alpha and beta) was **********(ed) with TAK1, it turned out that TAK1 and IKK (IKK alpha and beta) are interacting by intracellular.
[0126] However, in the cell in which TAB1 also carried out manifestation enhancement with TAK1 and IKK, IKK was not detected in the anti-flag immunoprecipitation fraction. Although IKK and stable association were produced from this in intracellular in the condition that TAK1 is not activated, where TAB1 is activated, it was thought that stable association with association with intracellular IKK was not seen.
[0127] Moreover, the inclination for the mobility in the SDS-polyacrylamide gel electrophoresis of IKK (IKK alpha and beta) to decrease a little in the cell in which TAB1 also carried out manifestation enhancement with TAK1 and IKK was accepted as a result of immunity blotting of the immunity extract of a cell solution. On the other hand, such an inclination was not seen in the cell which did not carry out manifestation enhancement of TAB1.
[0128] It was thought that both the subunits (IKK alpha and beta) of IKK received phosphorylation from these things by intracellular by existence of TAK1 activated by TAB1. That is, it is thought that TAK1 guides NF-kappa B activation like NIK (Regnier et al., 1997; Woronicz et al., 1997) by phosphorizing IKK (or molecule which interacts on IKK complex and a functional target), and promoting the kinase activity of IKK.
[0129] Example 9 As it was below activation of the IKK complex by TAK1, about the IKK complex which carried out immunoprecipitation from the cell which carried out manifestation enhancement of TAK1 with TAB1, the kinase reaction (IKK kinase assay) which makes IkappaB a substrate was carried out, and activation of IKK complex was detected.
[0130] (1) Transfection of the expression vector of the wild type TAK1 (Flag-TAK1) by which flag addition was carried out, or a variant TAK1 (Flag-TAK1K63W) was carried out to the HeLa cell with independent or TAB1 expression vector like the transfection of a cell and immunoprecipitation ****, and an example 5.
[0131] Moreover, by the system which carries out manifestation enhancement, the expression vector of IKK (Xpress-IKKalpha or Xpress-IKKbeta) by which Xpress tag addition was carried out as well as an example 8 added and carried out transfection of the foreignness IKK to coincidence.
[0132] From the cell 24 hours after transfection, like the example 6, the cell solution was prepared and immunoprecipitation was performed. In order to carry out immunoprecipitation of the internality IKK complex, for the immunoprecipitation of Foreignness IKK, the anti-Xpress antibody (M-21) (product made from Santa Cruz Biotechnology) was used for the antibody used for **** immunoprecipitation, using an anti-IKKalpha antibody (H-744) (product made from Santa Cruz Biotechnology). IKKbeta as well as IKKalpha recognizes the used anti-IKKalpha antibody.
[0133] (2) About the immunoprecipitation fraction obtained with the IKK kinase assay above, the in vitro kinase reaction was performed like the example 7. As a **** substrate, recombination IkappaB

(2.5microg) was added to the system of reaction. SDS-polyacrylamide gel electrophoresis was presented with reaction mixture after reaction termination, and autoradiography was carried out about the gel after migration.
[0134] The fusion peptide (the following, GST-IkappaB alpha1-54) which connected the partial polypeptide which becomes the C terminal of GST (glutathione-S-transferase) from the amino acid residue to the 1st to the 54th of Homo sapiens IkappaBalpha as recombination IkappaB made into a reaction substrate was used.
[0135] Recombination IkappaB was prepared from the culture of the transformant which introduced the expression vector of GST-IkappaB alpha1-54 into the Escherichia coli host. The expression vector of GST-IkappaB alpha1-54 inserted at least in the BamHI-EcoRI cutting section of vector plasmid pGEX-2T (product made from Pharmacia) the cDNA part which carries out the code of the amino acid residue to the 54th from from among [1st] cDNA(s) of Homo sapiens IkappaBalpha (Genbank/EMBL accession No.M69043;Cell, the 65th volume, the 1281 - 1289th page, 1991), and produced it.
[0136] The result of IKK kinase assay was shown in drawing 8 . (A) is as a result of the kinase assay of internality IKK complex (immunoprecipitation fraction by the anti-IKKalpha antibody), and (B) is as a result of the kinase assay of Foreignness IKK (immunoprecipitation fraction by the anti-Xpress antibody).
[0137] When manifestation enhancement of both the wild types TAK1 (Flag-TAK1) and TAB1 that carried out flag addition was carried out as shown in drawing 8 (A), the IKK kinase activity of internality IKK complex increased notably. On the other hand, the variant TAK1 (Flag-TAK1K63W) lacking in kinase activity did not promote IKK activity.
[0138] moreover, the case where manifestation enhancement of the wild type TAK1 is carried out with TAB1 also in the cell which made Foreignness IKK discover as shown in drawing 8 (B) -- Foreignness IKK -- although the IKK kinase activity of alpha and beta increased, even if it carried out manifestation enhancement with TAB1, IKK kinase activity did not increase by the variant TAK1.

[0139] TAK1 by which these results were activated by TAB1 proves that NF-kappa B is activated by activating IKKalpha and IKKbeta.
[0140] (3) An operation of the examined substance to the IKK complex activation by TAK1 can be authorized using the same system as the assay above of an operation of an examined substance. That is, the cell which carried out manifestation enhancement of TAK1 with TAB1 is obtained, and this is cultivated under existence of an examined substance or nonexistence. It judges whether about the cell after culture, immunoprecipitation of the IKK complex fraction is carried out like the above, the IKK kinase activity of an immunoprecipitation fraction is measured, and IKK kinase activity decreases by existence of an examined substance.
[0141]
[Effect of the Invention] The approach of this invention turns into the identification approach of NF-kappa B activation depressant and the screening approach of having focused on a new transfer molecule. According to this invention, the NF-kappa B activation depressant new type which has point of application in TAK1 can be obtained. Moreover, the approach of this invention is useful also as the identification approach of the remedy of diseases, such as an autoimmune disease and an intractable disease which presents an inflammation symptom, and/or a prophylactic, and the screening approach.
[0142] Since point of application of the drug [the drug chosen by the approach of this invention or] identified is clear, it is advantageous to the development as drugs.
[0143] Moreover, the drug which has the operation which checks or controls the function of TAK1 serves as NF-kappa B activation depressant new type, and also serves as a remedy of diseases, such as autoimmune diseases (rheumatoid arthritis, systemic lupus erythematosus, systemic scleroderma, Behcet's disease, a periarteritis nodosa, ulcerative colitis, glomerulonephritis, etc.), an intractable disease which presents an inflammation symptom, various viral diseases (the osteoarthritis, atherosclerosis, psoriasis, atopic dermatitis, etc.), endotoxin shock, and septicemia, and/or a prophylactic.

[0144]
[Layout Table] array number: — die-length [of one array]: — mold [of 30 arrays]: — number [of nucleic-acid chains]: — single strand topology: — a nucleic acid (synthetic primer) besides class: of a straight chain-like array
Array GGCCAGATCT ATGTCGACAG CCTCCGCCGC.
[0145] array number: — die-length [of two arrays]: — mold [of 30 arrays]: — number [of nucleic-acid chains]: — single strand topology: — a nucleic acid (synthetic primer) besides class: of a straight chain-like array
Array GCGCAGATCT TCATGAAGTG CCTTGTCGTT.
[0146]
array number: — die-length [of three arrays]: — mold [of 2785 arrays]: — number [of nucleic-acid chains]: — double strand topology: — class [of straight chain-like array]: — cDNA to mRNA array GGACACGGCT GTGGCCGCTG CCTCTACCCC CGCCACGGAT CGCCGGGTAG TAGGACTGCG 60 CGGCTCCAGG CTGAGGGTCG GTCCGGAGGC GGGTGGGCGC GGGTCTCACC CGGATTGTCC 120 GGGTGGCACC GTTCCCGGCC CCACCGGGCG CCGCGAGGGA TC 162 ATG TCT ACA GCC TCT GCC GCC TCC TCC TCC TCC TCG TCT TCG GCC 207 Met Ser Thr Ala Ser Ala Ala Ser Ser Ser Ser Ser SerSer Ala 1 5 10 15 GGT GAG ATG ATC GAA GCC CCT TCC CAG GTC CTC AAC TTT GAA GAG 252 Gly Glu Met Ile Glu Ala Pro Ser Gln Val Leu Asn Phe Glu Glu 20 25 30ATC GAC TAC AAG GAG ATC GAG GTG GAA GAG GTT GTT GGA AGA GGA 297 Ile Asp Tyr LysGlu Ile Glu Val Glu Glu Val Val Gly Arg Gly 35 40 45GCC TTT GGA GTT GTT TGC AAA GCT AAG TGG AGA GCAAAA GAT GTT342 Ala Phe Gly ValVal Cys Lys Ala Lys Trp Arg Ala Lys Asp Val 50 55 60 GCT ATT AAA CAA ATA GAA AGT GAA TCT GAGAGG AAA GCG TTT ATT 387 Ala Ile Lys Gln Ile Glu Ser Glu Ser Glu Arg Lys Ala Phe Ile 65 70 75 GTA GAG CTT CGG CAGTTA TCC CGT GTG AAC CAT CCT AAT ATT GTA 432 Val Glu Leu Arg Gln Leu Ser Arg Val Asn His Pro Asn Ile Val 80 85 90AAG CTT TAT GGA GCC TGC TTG AAT CCA GTGTGT CTT GTG ATG GAA 477 Lys Leu Tyr GlyAla Cys Leu Asn Pro Val Cys Leu Val Met Glu 95 100 105 TAT GCT GAA GGG GGC TCT TTA TAT AAT GTG CTG CAT GGT GCT GAA 522 Tyr Ala Glu Gly GlySer Leu Tyr Asn Val Leu His Gly Ala Glu 110 115 120 CCA TTG CCA TAT TATACT GCT GCC CAC GCAATGAGT TGG TGT TTA 567 Pro Leu ProTyr Tyr Thr Ala Ala His Ala Met Ser Trp Cys Leu 125 130 135 CAG TGT TCC CAA GGA GTG GCT TAT CTT CAC AGC ATG CAA CCC AAA 612 GlnCysSer Gln Gly Val Ala Tyr Leu His Ser Met GlnPro Lys 140 145 150GCG CTA ATT CAC AGG-GAC-CTG-AAA-CCA CCA AAC TTA CTG CTG-GTT 657Ala Leu Ile His Arg-Asp-Leu-Lys-Pro Pro Asn Leu Leu Leu-Val 155 160 165GCA GGG GGG ACA GTT CTA AAA ATT TGT GAT TTT GGT ACA GCC TGT 702 Ala Gly Gly Thr Val Leu Lys Ile Cys Asp Phe Gly Thr Ala Cys 170 175 180 GAC ATT CAG ACA CAC ATG ACC AAT AAC AAG GGG AGT GCT GCT TGG 747 Asp Ile Gln Thr His Met Thr Asn Asn Lys Gly Ser Ala Ala Trp 185 190 195 ATG GCA CCT GAA GTT TTT GAA GGT AGT AAT TAC AGT GAA AAA TGT 792 Met Ala Pro Glu Val Phe Glu Gly Ser Asn Tyr Ser Glu Lys Cys 200 205 210 GAC GTC TTC AGC TGG GGT ATT ATT CTT TGG GAA GTG ATA ACG CGT 837 Asp Val Phe Ser Trp Gly Ile Ile Leu Trp Glu Val Ile Thr Arg 215 220 225 CGG AAA CCC TTT GAT GAG ATT GGT GGC CCA GCT TTC CGA ATC ATG 882 Arg Lys Pro Phe Asp Glu Ile Gly Gly Pro Ala Phe Arg Ile Met 230 235 240 TGG GCT GTT CAT AAT GGT ACT CGA CCA CCA CTG ATA AAA AAT TTA 927 Trp Ala Val His Asn Gly Thr Arg Pro Pro Leu Ile Lys Asn Leu 245 250 255 CCT AAG CCC ATT GAG AGC CTG ATG ACT CGT TGT TGG TCT AAA GAT 972 Pro Lys Pro Ile Glu Ser Leu Met Thr Arg Cys Trp Ser Lys Asp 260 265 270 CCT TCC CAG CGC CCT TCA ATG GAG GAA ATT GTG AAA ATA ATG ACT 1017 Pro Ser Gln Arg Pro Ser Met Glu Glu Ile Val Lys Ile Met Thr 275 280 285 CAC TTG ATG CGG TAC TTT CCA GGA GCA GAT GAG CCA TTA CAG TAT 1062 His Leu Met Arg Tyr Phe Pro Gly Ala Asp Glu Pro Leu Gln Tyr 290 295 300 CCT TGT CAG TAT TCA GAT GAA GGA CAG AGC AAC TCT GCC ACC AGT 1107 Pro Cys Gln Tyr Ser Asp Glu Gly Gln Ser Asn Ser Ala Thr Ser 305 310 315 ACA GGC TCA TTC ATG GAC ATT GCT TCT ACA AAT ACG AGT AAC AAA 1152 Thr Gly Ser Phe Met Asp Ile Ala Ser Thr Asn Thr Ser Asn Lys 320 325 330 AGT GAC ACT AAT ATG GAG CAA GTT CCT GCC ACA AAT GAT ACT ATT 1197 Ser Asp Thr Asn Met Glu Gln Val Pro Ala Thr Asn Asp Thr Ile 335 340 345AAG CGC TTA GAA TCA-AAA-TTG-TTG-AAA AAT CAG GCA AAG CAA-CAG 1242Lys Arg

Leu Glu Ser Lys Leu Leu Lys-Asn-Gln-Ala-Lys-Gln-Gln 350 355 360AGT GAA TCT GGA CGT TTA AGC TTG GGA GCC TCC CGT GGG AGC-AGT 1287 Ser Glu Ser Gly Arg Leu Ser Leu Gly Ala Ser Arg Gly Ser Ser 365 370 375 GTG GAG AGC TTG CCC CCA ACC TCT GAG GGC AAG AGG ATG AGT GCT 1332 Val Glu Ser Leu Pro Pro Thr Ser Glu Gly Lys Arg Met Ser Ala 380 385 390 GAC ATG TCT GAA ATA GAA GCT AGG ATC GCC GCA ACC ACA GGC AAC 1377 Asp Met Ser Glu Ile Glu Ala Arg Ile Ala Ala Thr Thr Gly Asn 395 400 405 GGA CAG CCA AGA CGT AGA TCC ATC CAA GAC TTG ACT GTA ACT GGA 1422 Gly Gln Pro Arg Arg Arg Ser Ile Gln Asp Leu Thr Val Thr Gly 410 415 420 ACA GAA CCT GGT CAG GTG AGC AGT AGG TCA TCC AGT CCC AGT GTC 1467 Thr Glu Pro Gly Gln Val Ser Ser Arg Ser Ser Ser Pro Ser Val 425 430 435 AGA ATG ATT ACT ACC TCA GGA CCA ACC TCA GAA AAG CCA ACT CGA 1512 Arg Met Ile Thr Thr Ser Gly Pro Thr Ser Glu Lys Pro Thr Arg 440 445 450 AGT CAT CCA TGG ACC CCT GAT GAT TCC ACA GAT ACC AAT GGA TCA 1557 Ser His Pro Trp Thr Pro Asp Asp Ser Thr Asp Thr Asn Gly Ser 455 460 465 GAT AAC TCC ATC CCA ATG GCT TAT CTT ACA CTG GAT CAC CAA CTA 1602 Asp Asn Ser Ile Pro Met Ala Tyr Leu Thr Leu Asp His Gln Leu 470 475 480 CAG CCT CTA GCA CCG TGC CCA AAC TCC AAA GAA TCT ATG GCA GTG 1647 Gln Pro Leu Ala Pro Cys Pro Asn Ser Lys Glu Ser Met Ala Val 485 490 495 TTT GAA CAG CAT TGT AAA ATG GCA CAA GAA TAT ATG AAA GTT CAA 1692 Phe Glu Gln His Cys Lys Met Ala Gln Glu Tyr Met Lys Val Gln 500 505 510 ACA GAA ATT GCA TTG TTA TTA CAG AGA AAG CAA GAA CTA GTT GC A 1737Thr Glu Ile Ala Leu Leu Leu Gln Arg-Lys-Gln-Glu-Leu-Val-Ala 515 520 525GAA CTG GAC CAG GAT-GAA-AAG-GAC-CAG CAA AAT ACA TCT CGC-CTG 1782Glu Leu Asp Gln Asp Glu Lys Asp Gln-Gln-Asn-ThrSer Arg Leu 530 535 540 GTA CAG GAA CAT AAA AAG CTT TTA GAT GAA AAC AAA AGC CTT TCT 1827 Val Gln Glu His Lys Lys Leu Leu Asp Glu Asn Lys Ser Leu Ser 545 550 555 ACT TAC TAC CAG CAA TGC AAA AAA CAA CTA GAG GTC ATC AGA AGT 1872 Thr Tyr Tyr Gln Gln Cys Lys Lys Gln Leu Glu Val Ile Arg Ser 560 565 570 CAG CAG CAG AAA CGA CAA GGC ACT TCA 1899 Gln Gln Gln Lys Arg Gln Gly Thr Ser 575 579 TGATTCTCTG GGACCGTTAC ATTTTGAAAT ATGCAAAGAA AGACTTTTTT TTTAAGGAAA 1959 GGAAAACCTT ATAATGACGA TTCATGAGTG TTAGCTTTTT GGCGTGTTCT GAATGCCAAC 2019 TGCCTATATT TGCTGCATTT TTTTCATTGT TTATTTTCCT TTTCTCATGG TGGACATACA 2079 ATTTTACTGT TTCATTGCAT AACATGGTAG CATCTGTGAC TTGAATGAGC AGCACTTTGC 2139 AACTTCAAAA CAGATGCAGT GAACTGTGGC TGTATATGCA TGCTCATTGT GTGAAGGCTA 2199 GCCTAACAGA ACAGGAGGTA TCAAACTAGC TGCTATGTGC AAACAGCGTC CATTTTTTCA 2259 TATTAGAGGT GGAACCTCAA GAATGACTTT ATTCTTGTAT CTCATCTCAA AATATTAATA 2319 ATTTTTTTCC CAAAAGATGG TATATACCAA GTTAAAGACA GGGTATTATA AATTTAGAGT 2379 GATTGGTGGT ATATTACGGA AATACGGAAC CTTTAGGGAT AGTTCCGTGT AAGGGCTTTG 2439 ATGCCAGCAT CCTTGGATCA GTACTGAACT CAGTTCCATC CGTAAAATAT GTAAAGGTAA 2499 GTGGCAGCTG CTCTATTTAA TGAAAGCAGT TTTACCGGAT TTTGTTAGAC TAAAATTTGA 2559 TTGTGATACA TTGAACAAAA TGGAACTCAT TTTTTTTTAA GGAGTAAAGA TTTTTAATTC 2619 TGTGATTGTG TGTATGTGTG TTGAAACTGT AAAGCTTTTA TGACTCTAAT ATTAATCTCT 2679 TAAATGAAAT TAAAAGGCAA AAGAACATGA TTGAGCTTAA ATGATCATTT CTTCCTGCAG 2739 TGATTCTTGG ATTGTTTTCT CATGTATTTG AAAAAAAAAA AAAAAA 2785.

[0147]

array number: — die-length [of four arrays]: — mold [of 2866 arrays]: — number [of nucleic-acid chains]: — double strand topology: — class [of straight chain-like array]: — cDNA to mRNA array GGACACGGCT GTGGCCGCTG CCTCTACCCC CGCCACGGAT CGCCGGGTAG TAGGACTGCG 60 CGGCTCCAGG CTGAGGGTCG GTCCGGAGGC GGGTGGGCGC GGGTCTCACC CGGATTGTCC 120 GGGTGGCACC GTTCCCGGCC CCACCGGGCG CCGCGAGGGA TC 162 ATG TCT ACA GCC TCT GCC GCC TCC TCC TCC TCC TCG TCT TCG GCC 207 Met Ser Thr Ala Ser Ala Ala Ser Ser Ser Ser Ser SerSer Ala 1 5 10 15 GGT GAG ATG ATC GAA GCC CCT TCC CAG GTC CTC AAC TTT GAA GAG 252 Gly Glu Met Ile Glu Ala Pro Ser Gln Val Leu Asn Phe Glu Glu 20 25 30ATC GAC TAC AAG GAG ATC GAG GTG GAA GAG GTT GTT GGA AGA GGA 297 Ile Asp Tyr LysGlu Ile Glu Val Glu Glu Val Val Gly Arg Gly 35 40 45GCC TTT GGA GTT GTT TGC AAA GCT AAG TGG AGA GCAAAA GAT GTT342 Ala Phe Gly ValVal Cys Lys Ala Lys Trp Arg Ala Lys Asp Val 50 55 60 GCT ATT AAA CAA ATA GAA AGT GAA TCT

GAGAGG AAA GCG TTT ATT 387 Ala Ile Lys Gln Ile Glu Ser Glu Ser Glu Arg Lys Ala Phe Ile 65 70 75 GTA GAG CTT CGG CAGTTA TCC CGT GTG AAC CAT CCT AAT ATT GTA 432 Val Glu Leu Arg Gln Leu Ser Arg Val Asn His Pro Asn Ile Val 80 85 90AAG CTT TAT GGA GCC TGC TTG AAT CCA GTGTGT CTT GTG ATG GAA 477 Lys Leu Tyr GlyAla Cys Leu Asn Pro Val Cys Leu Val Met Glu 95 100 105 TAT GCT GAA GGG GGC TCT TTA TAT AAT GTG CTG CAT GGT GCT GAA 522 Tyr Ala Glu Gly GlySer Leu Tyr Asn Val Leu His Gly Ala Glu 110 115 120 CCA TTG CCA TAT TATACT GCT GCC CAC GCAATGAGT TGG TGT TTA 567 Pro Leu Pro Tyr Tyr Thr Ala Ala His Ala Met Ser Trp Cys Leu125 130 135 CAG TGT TCC CAA GGA GTG GCT TAT CTT CAC AGC ATG CAA CCC AAA 612 ProLeuPro Tyr Tyr Thr Ala Ala His Ala Met Ser TrpCys Leu 140 145 150GCG CTA ATT CAC AGG GAC CTG AAA CCA CCA AACTTA CTG CTG GTT 657 Ala Leu Ile His Arg Asp Leu Lys Pro Pro Asn Leu Leu Leu Val155 160 165 GCA GGG GGG ACA GTT CTA AAA ATT TGT GAT TTT GGT ACA GCCTGT 702 Ala Gly Gly Thr Val Leu Lys Ile Cys Asp Phe Gly Thr Ala Cys 170 175 180GAC ATT CAG ACA CAC-ATG-ACC-AAT-AAC AAG GGG AGT GCT GCT-TGG 747Asp Ile Gln Thr His-Met-Thr-Asn-Asn Lys Gly Ser Ala Ala-Trp 185 190 195ATG GCA CCT GAA GTT TTT GAA GGT AGT AAT TAC AGT GAA AAA TGT 792 Met Ala Pro Glu Val Phe Glu GlySer Asn Tyr Ser Glu Lys Cys 200 205 210 GAC GTC TTC AGC TGG GGT ATT ATT CTT TGG GAA GTG ATA ACG CGT 837 Asp Val Phe Ser Trp Gly Ile Ile Leu Trp Glu Val Ile Thr Arg 215 220 225 CGG AAA CCC TTT GAT GAG ATT GGT GGC CCA GCT TTC CGA ATC ATG 882 Arg Lys Pro Phe Asp Glu Ile Gly Gly Pro Ala Phe Arg Ile Met 230 235 240 TGG GCT GTT CAT AAT GGT ACT CGA CCA CCA CTG ATA AAA AAT TTA 927 Trp Ala Val His Asn Gly Thr Arg Pro Pro Leu Ile Lys Asn Leu 245 250 255 CCT AAG CCC ATT GAG AGC CTG ATG ACT CGT TGT TGG TCT AAA GAT 972 Pro Lys Pro Ile Glu Ser Leu Met Thr Arg Cys Trp Ser Lys Asp 260 265 270 CCT TCC CAG CGC CCT TCA ATG GAG GAA ATT GTG AAA ATA ATG ACT 1017 Pro Ser Gln Arg Pro Ser Met Glu Glu Ile Val Lys Ile Met Thr 275 280 285 CAC TTG ATG CGG TAC TTT CCA GGA GCA GAT GAG CCA TTA CAG TAT 1062 His Leu Met Arg Tyr Phe Pro Gly Ala Asp Glu Pro Leu Gln Tyr 290 295 300 CCT TGT CAG TAT TCA GAT GAA GGA CAG AGC AAC TCT GCC ACC AGT 1107 Pro Cys Gln Tyr Ser Asp Glu Gly Gln Ser Asn Ser Ala Thr Ser 305 310 315 ACA GGC TCA TTC ATG GAC ATT GCT TCT ACA AAT ACG AGT AAC AAA 1152 Thr Gly Ser Phe Met Asp Ile Ala Ser Thr Asn Thr Ser Asn Lys 320 325 330 AGT GAC ACT AAT ATG GAG CAA GTT CCT GCC ACA AAT GAT ACT ATT 1197 Ser Asp Thr Asn Met Glu Gln Val Pro Ala Thr Asn Asp Thr Ile 335 340 345 AAG CGC TTA GAA TCA AAA TTG TTG AAA AAT CAG GCA AAG CAA CAG 1242 Lys Arg Leu Glu Ser Lys Leu Leu Lys Asn Gln Ala Lys Gln Gln 350 355 360 AGT GAA TCT GGA CGT TTA AGC-TTG-GGA-GCC-TCC CGT GGG AGC AGT 1287Ser Glu Ser Gly Arg Leu Ser Leu Gly-Ala-Ser-Arg-Gly-Ser-Ser 365 370 375GTG GAG AGC TTG CCC-CCA-ACC-TCT-GAG GGC AAG AGG ATG AGT-GCT 1332Val Glu Ser L eu Pro Pro Thr Ser Glu Gly Lys Arg Met Ser Ala 380 385 390 GAC ATG TCT GAA ATA GAA GCT AGG ATC GCC GCA ACC ACA GCC TAT 1377 Asp Met Ser Glu Ile Glu Ala Arg Ile Ala Ala Thr Thr Ala Tyr 395 400 405 TCC AAG CCT AAA CGG GGC CAC CGT AAA ACT GCT TCA TTT GGC AAC 1422 Ser Lys Pro Lys Arg Gly His Arg Lys Thr Ala Ser Phe Gly Asn 410 415 420 ATT CTG GAT GTC CCT GAG ATC GTC ATA TCA GGC AAC GGA CAG CCA 1467 Ile Leu Asp Val Pro Glu Ile Val Ile Ser Gly Asn Gly Gln Pro 425 430 435 AGA CGT AGA TCC ATC CAA GAC TTG ACT GTA ACT GGA ACA GAA CCT 1512 Arg Arg Arg Ser Ile Gln Asp Leu Thr Val Thr Gly Thr Glu Pro 440 445 450 GGT CAG GTG AGC AGT AGG TCA TCC AGT CCC AGT GTC AGA ATG ATT 1557 Gly Gln Val Ser Ser Arg Ser Ser Ser Pro Ser Val Arg Met Ile 455 460 465 ACT ACC TCA GGA CCA ACC TCA GAA AAG CCA ACT CGA AGT CAT CCA 1602 Thr Thr Ser Gly Pro Thr Ser Glu Lys Pro Thr Arg Ser His Pro 470 475 480 TGG ACC CCT GAT GAT TCC ACA GAT ACC AAT GGA TCA GAT AAC TCC 1647 Trp Thr Pro Asp Asp Ser Thr Asp Thr Asn Gly Ser Asp Asn Ser 485 490 495 ATC CCA ATG GCT TAT CTT ACA CTG GAT CAC CAA CTA CAG CCT CTA 1692 Ile Pro Met Ala Tyr Leu Thr Leu Asp His Gln Leu Gln Pro Leu 500 505 510 GCA CCG TGC CCA AAC TCC AAA GAA TCT ATG GCA GTG TTT GAA CAG 1737 Ala Pro Cys Pro Asn Ser Lys Glu Ser Met Ala Val Phe Glu Gln 515 520 525 CAT TGT AAA ATG GCA CAA GAA TAT ATG AAA GTT CAA ACA GAA ATT 1782 His Cys Lys Met Ala Gln Glu Tyr Met Lys Val Gln Thr Glu Ile 530 535 540GCA TTG TTA TTA CAG AGA AAG CAA GAA CTA GTT GCA GAA CTG GAC 1827Ala Leu Leu Leu Gln Arg Lys Gln Glu-Leu-Val-Ala-Glu-Leu-Asp 545 550 555CAG

GAT GAA AAG GAC-CAG-CAA-AAT-ACA TCT CGC CTG GTA CAG-GAA 1872Gln Asp Glu Lys Asp Gln Gln Asn Thr Ser Arg Leu Val Gln Glu 560 565 570 CAT AAA AAG CTT TTA GAT GAA AAC AAA AGC CTT TCT ACT TAC TAC 1917 His Lys Lys Leu Leu Asp Glu Asn Lys Ser Leu Ser Thr Tyr Tyr 575 580 585 CAG CAA TGC AAA AAA CAA CTA GAG GTC ATC AGA AGT CAG CAG CAG 1962 Gln Gln Cys Lys Lys Gln Leu Glu Val Ile Arg Ser Gln Gln Gln 590 595 600 AAA CGA CAA GGCACT TCA 1980 Lys Arg Gln Gly ThrSer 605 606 TGATTCTCTG GGACCGTTAC ATTTTGAAAT ATGCAAAGAA AGACTTTTTT TTTAAGGAAA 2040 GGAAAACCTT ATAATGACGA TTCATGAGTG TTAGCTTTTT GGCGTGTTCT GAATGCCAAC 2100 TGCCTATATT TGCTGCATTT TTTTCATTGT TTATTTTCCT TTTCTCATGG TGGACATACA 2160 ATTTTACTGT TTCATTGCAT AACATGGTAG CATCTGTGAC TTGAATGAGC AGCACTTTGC 2220 AACTTCAAAA CAGATGCAGT GAACTGTGGC TGTATATGCA TGCTCATTGT GTGAAGGCTA 2280 GCCTAACAGA ACAGGAGGTA TCAAACTAGC TGCTATGTGC AAACAGCGTC CATTTTTTCA 2340 TATTAGAGGT GGAACCTCAA GAATGACTTT ATTCTTGTAT CTCATCTCAA AATATTAATA 2400 ATTTTTTTCC CAAAAGATGG TATATACCAA GTTAAAGACA GGGTATTATA AATTTAGAGT 2460 GATTGGTGGT ATATTACGGA AATACGGAAC CTTTAGGGAT AGTTCCGTGT AAGGGCTTTG 2520 ATGCCAGCAT CCTTGGATCA GTACTGAACT CAGTTCCATC CGTAAAATAT GTAAAGGTAA 2580 GTGGCAGCTG CTCTATTTAA TGAAAGCAGT TTTACCGGAT TTTGTTAGAC TAAAATTTGA 2640 TTGTGATACA TTGAACAAAA TGGAACTCAT TTTTTTTTAA GGAGTAAAGA TTTTTAATTC 2700 TGTGATTGTG TGTATGTGTG TTGAAACTGT AAAGCTTTTA TGACTCTAAT ATTAATCTCT 2760 TAAATGAAAT TAAAAGGCAA AAGAACATGA TTGAGCTTAA ATGATCATTT CTTCCTGCAG 2820 TGATTCTTGG ATTGTTTTCT CATGTATTTG AAAAAAAAAA AAAAAA 2866.

[0148]

array number: -- die-length [of five arrays]: -- mold [of 1704 arrays]: -- number [of nucleic-acid chains]: -- double strand topology: -- class [of straight chain-like array]: -- cDNA to mRNA array ATG TCT ACA GCC TCT GCC GCC TCC TCC TCC TCC TCG TCT TCG GCC 45 Met Ser Thr Ala Ser Ala Ala Ser Ser Ser Ser Ser Ser Ser Ala 1 5 10 15 GGT GAG ATG ATC GAA GCC CCT TCC CAG GTC CTC AAC TTT GAA GAG 90 Gly Glu Met Ile Glu Ala Pro Ser Gln Val Leu Asn Phe Glu Glu 20 25 30 ATC GAC TAC AAG GAGATC GAG GTG GAA GAGGTT GTT GGA AGA GGA 135 Ile Asp Tyr Lys Glu Ile Glu Val Glu Glu Val Val Gly Arg Gly 35 40 45 GCC TTT GGA GTT GTTTGC AAA GCT AAG TGGAGA GCA AAA GAT GTT 180 Ala Phe Gly ValVal Cys Lys Ala Lys Trp Arg Ala Lys Asp Val 50 55 60 GCT ATT AAA CAA ATA GAA AGT GAATCT GAG AGG AAA GCG TTT ATT 225 Ala Ile Lys Gln Ile Glu Ser Glu Ser Glu Arg Lys Ala Phe Ile 65 70 75 GTA GAG CTT CGG CAGTTA TCC CGT GTG AAC CAT CCT AAT ATT GTA 270 Val Glu Leu Arg Gln Leu Ser Arg Val Asn His Pro Asn Ile Val 80 85 90AAG CTT TAT GGA GCC TGC TTG AAT CCA GTGTGT CTT GTG ATG GAA 315 Lys Leu Tyr GlyAla Cys Leu Asn Pro Val Cys Leu Val Met Glu 95 100 105 TAT GCT GAA GGG GGC TCT TTA TAT AAT GTG CTG CAT GGT GCT GAA 360 Tyr Ala Glu Gly GlySer Leu Tyr Asn Val Leu His Gly Ala Glu 110 115 120 CCA TTG CCA TAT TATACT GCT GCC CAC GCAATGAGT TGG TGT TTA 405 Pro Leu Pro Tyr Tyr Thr Ala Ala His Ala Met Ser Trp Cys Leu125 130 135 CAG TGT TCC CAA GGA GTG GCT TAT CTT CAC AGC ATG CAA CCC AAA 450 GlnCysSer Gln Gly Val Ala Tyr Leu His Ser Met GlnPro Lys 140 145 150GCG CTA ATT CAC AGG GAC CTG AAA CCA CCA AACTTA CTG CTG GTT 495 Ala Leu Ile His Arg Asp Leu Lys Pro Pro Asn Leu Leu Leu Val155 160 165 GCA GGG GGG ACA GTT CTA AAA ATT TGT GAT TTT GGT ACA GCCTGT 540 Ala Gly Gly Thr Val Leu Lys Ile Cys Asp Phe Gly Thr Ala Cys 170 175 180 GAC ATT CAG ACA CAC ATG ACC AAT AAC AAG GGG AGT GCT GCT TGG 585 Asp Ile Gln Thr His Met Thr Asn Asn Lys Gly Ser Ala Ala Trp 185 190 195ATG GCA CCT GAA GTT-TTT-GAA-GGT-AGT AAT TAC AGT GAA AAA-TGT 630Met Ala Pro Glu Val-Phe-Glu-Gly-Ser Asn Tyr Ser Glu Lys-Cys 200 205 210GAC GTC TTC AGC TGG GGT ATT ATT CTT TGG GAA GTG ATA ACG CGT 675 Asp Val Phe Ser Trp Gly Ile Ile Leu Trp Glu Val Ile Thr Arg 215 220 225 CGG AAA CCC TTT GAT GAG ATT GGT GGC CCA GCT TTC CGA ATC ATG 720 Arg Lys Pro Phe Asp Glu Ile Gly Gly Pro Ala Phe Arg Ile Met 230 235 240 TGG GCT GTT CAT AAT GGT ACT CGA CCA CCA CTG ATA AAA AAT TTA 765 Trp Ala Val His Asn Gly Thr Arg Pro Pro Leu Ile Lys Asn Leu 245 250 255 CCT AAG CCC ATT GAG AGC CTG ATG ACT CGT TGT TGG TCT AAA GAT 810 Pro Lys Pro Ile Glu Ser Leu Met Thr Arg Cys Trp Ser Lys Asp 260 265

270 CCT TCC CAG CGC CCT TCA ATG GAG GAAATT GTG AAA ATA ATG ACT 855 Pro Ser Gln Arg Pro Ser Met Glu Glu Ile Val Lys Ile Met Thr 275 280 285 CAC TTG ATG CGG TAC TTT CCA GGA GCA GAT GAG CCA TTA CAG TAT 900 His Leu Met Arg Tyr Phe Pro Gly Ala Asp Glu Pro Leu Gln Tyr 290 295 300 CCT TGT CAG TAT TCA GAT GAA GGA CAG AGC AAC TCT GCC ACC AGT 945 Pro Cys Gln Tyr Ser Asp Glu Gly Gln Ser Asn Ser Ala Thr Ser 305 310 315 ACA GGC TCA TTC ATG GAC ATT GCT TCT ACA AAT ACG AGT AAC AAA 990 Thr Gly Ser Phe Met Asp Ile Ala Ser Thr Asn Thr Ser Asn Lys 320 325 330 AGT GAC ACT AAT ATG GAG CAA GTT CCT GCC ACA AAT GAT ACT ATT 1035 Ser Asp Thr Asn Met Glu Gln Val Pro Ala Thr Asn Asp Thr Ile 335 340 345 AAG CGC TTA GAA TCA AAA TTG TTG AAA AAT CAG GCA AAG CAA CAG 1080 Lys Arg Leu Glu Ser Lys Leu Leu Lys Asn Gln Ala Lys Gln Gln 350 355 360 AGT GAA TCT GGA CGT TTA AGC TTG GGA GCC TCC CGT GGG AGC AGT 1125 Ser Glu Ser Gly Arg Leu Ser Leu Gly Ala Ser Arg Gly Ser Ser 365 370 375 GTG GAG AGC TTG CCC CCA ACC TCT GAG GGC AAG AGG ATG AGT GCT 1170 Val Glu Ser Leu Pro Pro Thr Ser Glu Gly Lys Arg Met Ser Ala 380 385 390 GAC ATG TCT GAA ATA GAA GCT-AGG-ATC-GCC-GCA ACC ACA GCC TAT 1215Asp Met Ser Glu Ile Glu Ala Arg Ile-Ala-Ala-Thr-Thr-Ala-Tyr 395 400 405TCC AAG CCT AAA CGG-GGC-CAC-CGT-AAA ACT GCT TCA TTT GGC-AAC 1260Ser Lys Pro L ys Arg Gly His Arg Lys Thr Ala Ser Phe Gly Asn 410 415 420 ATT CTG GAT GTC CCT GAG ATC GTC ATA TCA GGC AAC GGA CAG CCA 1305 Ile Leu Asp Val Pro Glu Ile Val Ile Ser Gly Asn Gly Gln Pro 425 430 435 AGA CGT AGA TCC ATC CAA GAC TTG ACT GTA ACT GGA ACA GAA CCT 1350 Arg Arg Arg Ser Ile Gln Asp Leu Thr Val Thr Gly Thr Glu Pro 440 445 450 GGT CAG GTG AGC AGT AGG TCA TCC AGT CCC AGT GTC AGA ATG ATT 1395 Gly Gln Val Ser Ser Arg Ser Ser Ser Pro Ser Val Arg Met Ile 455 460 465 ACT ACC TCA GGA CCA ACC TCA GAA AAG CCA ACT CGA AGT CAT CCA 1440 Thr Thr Ser Gly Pro Thr Ser Glu Lys Pro Thr Arg Ser His Pro 470 475 480 TGG ACC CCT GAT GAT TCC ACA GAT ACC AAT GGA TCA GAT AAC TCC 1485 Trp Thr Pro Asp Asp Ser Thr Asp Thr Asn Gly Ser Asp Asn Ser 485 490 495 ATC CCA ATG GCT TAT CTT ACA CTG GAT CAC CAA CTA CAG CAA GAA 1530 Ile Pro Met Ala Tyr Leu Thr Leu Asp His Gln Leu Gln Gln Glu 500 505 510 CTA GTT GCA GAA CTG GAC CAG GAT GAA AAG GAC CAG CAA AAT ACA 1575 Leu Val Ala Glu Leu Asp Gln Asp Glu Lys Asp Gln Gln Asn Thr 515 520 525 TCT CGC CTG GTA CAG GAA CAT AAA AAG CTT TTA GAT GAA AAC AAA 1620 Ser Arg Leu Val Gln Glu His Lys Lys Leu Leu Asp Glu Asn Lys 530 535 540 GGC CTT TCT ACT TAC TAC CAG CAA TGC AAA AAA CAA CTA GAG GTC 1665 Gly Leu Ser Thr Tyr Tyr Gln Gln Cys Lys Lys Gln Leu Glu Val 545 550 555 ATC AGA AGT CAG CAG CAG AAA CGA CAA GGC ACT TCA TGA 1704 Ile Arg Ser Gln Gln Gln Lys Arg Gln Gly Thr Ser 560 565 567.

[0149] array number: — die-length [of six arrays]: — mold [of 30 arrays]: — number [of nucleic-acid chains]: — single strand topology: — a nucleic acid (synthetic primer) besides class: of a straight chain-like array

Array TTCCAAGCTT ATGGCGGCGC AGAGGAGGAG.

[0150] array number: — die-length [of seven arrays]: — mold [of 30 arrays]: — number [of nucleic-acid chains]: — single strand topology: — a nucleic acid (synthetic primer) besides class: of a straight chain-like array

Array TCCGGAATTC CTACGGTGCT GTCACCACGC

[Translation done.]